"Synthesis and structural studies of oligonucleotides with tRNA anticodon stem-loop sequences containing newly discovered modified nucleosides: ct^6A, ms^2ct^6A, ges^2U"

The presence of numerous modified nucleoside units is a unique feature of transfer nucleic acids (tRNA) structure. These tRNA modifications play a key role in protein biosynthesis, while also being involved in cellular regulatory pathways and stress-induced mechanisms. The state of tRNA modifications can be also correlated with various pathological conditions in the cells. Over a hundred different modified nucleosides have been already identified in tRNA. They are most commonly localized in the anticodon loop - the structural domain of tRNA directly involved in the process of decoding genetic information.

In the course of this study we will concentrate our attention on three among the most recently discovered and interesting tRNA modifications, namely: the cyclic N^6-threonylcarbamoyladenosine (**ct^6A**), cyclic 2-methylthio-N^6-threonylcarbamoyladenosine (**ms^2ct^6A**) and *S*-geranyl-2-tiouridine (**ges^2U**).

Cyclic nucleosides, **ct^6A** and **ms^2ct^6A**, are formed *in vivo* through enzymatic dehydration of linear **t^6A** and **ms^2t^6A**, highly conserved units localized at position 37 of tRNAs, known for more than 40 years. This transformation has important structural consequences, as the chemical nature of **ct^6A** and **ms^2ct^6A** makes them unable to adopt a planar conformation, which is characteristic for their linear counterparts. It was indeed this planar arrangement that, up to now, has been considered the key feature defining the biological role of these modifications. Consequently, the research conducted in the current project aims at understanding how the new cyclic and non-planar **ct^6A** and **ms^2ct^6A** modifications of the anticodon loop influence the structure and function of tRNA.

The *S*-geranyl-2-tiouridine (**ges^2U**), recently identified in the first anticodon position of bacterial tRNAs, constitutes an equally interesting and unusual tRNA modification. Its structure contains a bulky and lipophilic geranyl group – an element without precedent in the tRNA modifications known up to date. In this project we will study how the presence of such a bulky substituent alters the structure of the anticodon loop.

The main objective of our study is the chemical synthesis of a series of oligonucleotides derived from the natural tRNA anticodon stem-loops containing **ct^6A**, **ms^2ct^6A** and **ges^2U**, followed by their extensive structural characterization. We believe that our study will allow to gain important insights into how the unique structural motifs present in **ct^6A**, **ms^2ct^6A** and **ges^2U** influence the conformation of the anticodon loop and in consequence the functions of this crucial structural domain of tRNA.